271. (New) A method to activate expression of ~~a~~ an endogenous gene in an isolated eukaryotic cell comprising introducing a vector construct into said isolated eukaryotic cell, said vector construct comprising in operable combination: 1) a promoter; 2) an exon sequence located 3′ from and expressed by said promoter, said exon being derived from a naturally-occurring eukaryotic gene and not being a screenable marker gene; and 3) a splice donor sequence defining the 3′ region of said exon, said splice donor sequence being derived from a naturally-occurring eukaryotic gene; wherein said vector construct is non-homologously incorporated into the genome of said isolated ~~a~~ eukaryotic ~~target~~ cell and said splice donor sequence of the transcript encoded by said exon is spliced to a splice acceptor sequence of said endogenous ~~cellularly-encoded~~ gene.

REMARKS

New claim 271 has been submitted to avoid any question of compliance with 35 U.S.C. § 135(b). This claim has been written to copy, using the language of the present application, claim 15 of U.S. Patent No. 6,080,576, issued to Zambrowicz, et al. on June 27, 2000, from U.S. Patent Application No. 09/057,328, filed April 8, 1998, which is based on U.S. Provisional Application No. 60/079,729, filed March 27, 1998.

The remaining information required by 37 C.F.R. § 1.607 will be submitted in due course.

SUPPORT

Representative Support in Applicants' Earliest Priority U.S. Application No. 08/941,223

Support for activating expression can be found *inter alia* in the Abstract; page 24, lines 20–21; and original claim 61.

Regarding the recitation of "activate" and not "alter," whereas this term is used in original claim 18 for U.S. Patent No. 6,080,576, there is no specific discussion or definition of this term in that specification. However, there are many instances of over-expressing or activating a gene. Moreover, the person of ordinary skill in the art would have understood that when the 3´ gene traps affect gene expression, it is by activation of a gene. Accordingly, the term "alter" in claim 15 of the '576 patent is synonymous with the term "activation."

Support for expression in isolated eukaryotic cells can be found *inter alia* on page 7, line 23; page 8, line 9; page 30, lines 3, 4, 10, 13–17 and 27–28; page 31, line 8; and page 32, lines 10–25. On page 30, lines 13–17, it is also indicated that cells can be isolated from an animal. On page 30, line 27, it is indicated that cells are derived from any vertebrate tissue. On page 32, lines 19 and 20, the specification refers to introducing the construct by electroporation or liposome-mediated introduction. The methods would be practiced on cells not in contact with other cells. Moreover, since cells are cultured *in vitro*, the person of ordinary skill in the art would have recognized from the Applicants' specification that the vector is introduced into

single cells in isolation from one another in culture, and it is in the isolated cell that gene activation occurs.

In the specification for U.S. Patent No. 6,080,576, there is no specific disclosure of an isolated cell. However, the vectors are used to transfect cells in culture, and this could be viewed as cells that are separated from other cells or isolated. Further support for the use of isolated cells is found on page 6, line 26, of the application for the '576 patent in that the vector can be introduced by electroporation, microinjection, lipofection or transfection, which implies individual isolated cells.

Support for introducing the vector construct into the cell is a basic concept of the Applicants' invention and, therefore, is found throughout the specification. See for example, page 8, lines 5–9. For use of the term "vector," which is also found throughout the specification, please see, for example, page 22, lines 4–12.

Claim 15 in the '576 patent refers to a 3′ gene trap cassette. The Applicants' specification does not explicitly recite the term "3′ gene trap cassette." However, the art-recognized meaning of the term 3′ gene trap is a construct that, acting 5′ of a gene sequence, traps that gene sequence as a fusion transcript, where the fusion transcript contains both vector sequences and sequences from the trapped gene. Accordingly, many of Applicants' disclosed vectors would be recognized by the person of ordinary skill in the art as 3′ gene trap vectors. In particular, anything with a transcriptional regulatory sequence operably linked to a splice donor

will act as a 3′ gene trap. Pages 19–21, line 6, of the Applicants' specification include various examples of 3′ gene trap constructs. For other examples of 3′gene traps, please see Figures 1–4 and the figure legends on page 10, line 27 through page 11, line 21.

Support for the components on the vector being in operable combination can be found *inter alia* in Figures 1–4 and the figure legends on page 10, line 27 through page 11, line 21; page 9, lines 24–25; page 17, lines 21–30 through page 18, line 2; pages 19–21, line 6; page 25, line 17; and page 26, lines 9–23.

Support for the transcriptional regulatory sequence being a promoter can be found *inter alia* on page 10, lines 14–15.

Support for the exon being located 3′ from and expressed by the promoter can be found *inter alia* in Figures 1–4; page 17, lines 21–30 through page 18, line 2; pages 19–21, line 6; page 25, line 17; and page 26, lines 10–12.

Support for the exon being derived from a naturally-occurring eukaryotic gene can be found in the paragraph spanning pages 25–26 and in Figure 1.

The limitation in claim 15 of U.S. Patent No. 6,080,576, wherein the exon does not encode antibiotic resistance does not appear in Applicants' submitted claim 271. Applicants point out that no eukaryotic gene would confer antibiotic resistance. Indeed, Applicants note that

the phrase "said exon being derived from a naturally-occurring eukaryotic gene" was added to overcome art cited by the Examiner, whereas the phrase "said exon not encoding an activity conferring antibiotic resistance" was in the original claim. The cited art was relied upon as teaching that the exon could encode various markers, including markers not encoding antibiotic resistance. Accordingly, to overcome the art, the Applicants added the limitation that the exon is derived from a naturally-occurring eukaryotic gene. Addition of this new limitation made the prior limitation that the exon does not encode antibiotic resistance (disclosed in the '576 patent at 7:31–37) superfluous. This limitation, therefore, does not alter the scope of the claim and is omitted from claim 271.

Support for the exon not being a screenable marker can be found on page 25, line 30 through page 26, line 2; page 26, line 30 through page 27, line 2; page 28, lines 5–12, 14–16 and 24–27.

Support for the splice donor sequence defining the 3′ region of the exon can be found *inter alia* on page 26, lines 2–3.

Support for the splice donor sequence being derived from a naturally-occurring eukaryotic gene is found on page 27, lines 4–9.

The consensus splice donor sequence is by definition eukaryotic because splicing does not occur in prokaryotic cells. This consensus sequence provides for naturally-occurring splice donor sequences.

In the application for U.S. Patent No. 6,080,576, there is no explicit disclosure of the splice donor sequence having been derived from a naturally-occurring eukaryotic gene. However, there are instances where such splice donors are used. For example, on page 25, the text states that a 3′ gene trap cassette was constructed that replaced the exon and splice donor in an older 3′ gene trap vector with a naturally-occurring mouse exon that has the native splice donor sequence.

Support for the limitation, wherein the vector construct is non-homologously incorporated into the genome of the eukaryotic target cell, can be found *inter alia* in Applicants' specification on page 12, lines 5–21; page 14, lines 29–30 through page 15, line 24; page 15, lines 28–30 through page 16, line 4; page 27, lines 12–14; and original claim 34

Support for the limitation, wherein the splice donor sequence of the transcript encoded by the exon is spliced to a splice acceptor sequence of the cellularly-encoded gene, can be found *inter alia* on page 27, lines 10–18.

Representative Support in U.S. Applicants' Latest Specification: U.S. Application No. 09/276,820

Support for activating expression of a gene is a fundamental aspect of the invention and, therefore, is found throughout the application. See for example, the Abstract and page 45, lines 24–25.

Support for the claim being directed to an isolated cell can be found in the specification *inter alia* on page 10, lines 1 and 15–21; page 14, lines 28–30; page 53, lines 8–11 and 20–24; page 54, lines 11–12 and 24–25; and page 56, lines 3–7.

Support for the cell being eukaryotic can be found on page 10, lines 2–6; page 14, lines 28–30; and page 53, line 17.

Support for introducing a vector construct can be found in the specification *inter alia* in Figures 1–4 and in the figure legends on page 17, lines 10–28 through page 18, line 4; and page 42, lines 23–30 through page 43, line 3.

Support for the components in the vector construct being in operable combination are found in Figures 1–4 and in the figure legends on page 17, line 10 through page 18, line 4; page 6, line 18 through page 7, line 2; page 38, line 18 through page 40, line 25; page 46, line 25; and page 47, line 17 through page 48, line 2.

Support for a promoter can be found *inter alia* on page 7, lines 11–12.

Support for the exon 3′ from and expressed by the promoter can be found *inter alia* in Figures 1–4; page 37, lines 8–19; page 38, line 18 through page 40, line 25; page 46, line 25; and page 47, lines 17–20.

Support for the exon being derived from a naturally-occurring eukaryotic gene can be found *inter alia* in the paragraph spanning pages 46–47 and in Figure 1.

Support for the exon not being a screenable marker can be found on page 47, lines 8–10; page 48, lines 9–11; page 49, lines 14–21 and 23–25; and page 50, lines 3–5.

Support for a splice donor sequence defining the 3′ region of the exon can be found on page 47, lines 10–11.

Support for the splice donor sequence being derived from a naturally-occurring eukaryotic gene can be found on page 48, lines 13–18.

Support for the vector construct being non-homologously incorporated into the genome of the eukaryotic target cell can be found in the specification on page 30, lines 21–27; page 34, line 5 through page 35, line 17; and page 48, lines 21–24.

Support for the splice donor sequence of the transcript being spliced to the splice acceptor sequence of the cellularly-encoded gene can be found on page 48, lines 19–27.

Accordingly, no new matter has been added with Applicants' new claim.

Respectfully submitted,

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